FINAL REPORT FOR CDFA CONTRACT NUMBER 07-0176

IMPROVED DETECTION, MONITORING AND MANAGEMENT OF THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
Efficient and precise methods for detection of new colony infestations and for monitoring glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (GWSS) populations are lacking. This proposal addressed detection and monitoring methods and accompanying leafhopper behavior toward improved management of GWSS.

LAYPERSON SUMMARY
Management of the vector GWSS and Pierce's Disease is contingent on the availability of efficient field-sampling methods. This proposal aimed to improve upon the current monitoring methods. GWSS behavior in response to various types of traps in combination with host plants and other factors with potential to increase trap efficiency were investigated. Current trapping relies on use of the yellow Seabright trap which is flat with two sides covered with stickem to capture the insects. The trap attracts in two directions and has a total yellow attraction area of 653.8 cm^2 and a trapping surface area (stickem covered) of 409.4 cm^2. We have found that a yellow cylinder (tube) trap 7.6 × 30.5 cm (3 × 12 in, 730 cm^2 area) that samples in all directions (360^0) usually improves trap capture rate by 2-4 times for males and somewhat less for females. We have used Glidden Alkyd Industrial Enamel 4540, Safety Yellow, as the standard color and Tangletrap™ (Gemplers.com) as the standard sticky substance. Trap capture efficiency is inversely proportional to the distance from a host plant. GWSS respond to other leafhoppers when searching for and landing on a host plant and apparently in response to traps. Adding a leafhopper or model of a leafhopper to a trap can increase trap catch by 20-50% under low vector populations. Trap capture efficiency does not correlate well to leafhopper numbers found on host plants with the exception of within large blocks of citrus (Castle and Naranjo 2008), and may be inversely related on host plants with high nutritional quality such as crape myrtle when the plant is at peak quality with respect to xylem nutrients.

INTRODUCTION
The GWSS as a vector of *Xylella fastidiosa*, remains a threat to grapes, almonds, stone fruit and oleander and impacts citrus and nursery crops throughout much of California. It remains an important quarantine pest for the Napa and Sonoma Valleys and other critical uninfested locations. Due to the unique biology and behavior of GWSS which is driven by plant xylem chemistry and nutrition, conventional detection and monitoring approaches may not provide the necessary statistical precision needed by the regulatory and producer community for management decisions. This proposal addressed the detection and monitoring needs.

OBJECTIVES
Overall: To determine the most efficient and cost effective trapping system to detect and monitor *Homalodisca vitripennis* Germar (GWSS) population dynamics and the potential to manage GWSS populations.

1. Evaluate and summarize previous sampling and trapping efforts for GWSS.
2. Trap configuration and number: Determine the potential and optimize the number of traps that are most efficient and cost effective in detecting and estimating GWSS populations.
3. Determine the effects of host plants in combination with traps: Determine the potential and the optimization of a combination of GWSS host plants in sentinel plots to detect, estimate and manage GWSS population dynamics.

RESULTS AND DISCUSSION

A series of data are provided to indicate some of the approaches we have undertaken. In brief, we have looked at trap size, color, height, shape, orientation, background contrast, placement relative to vegetation, distance from vegetation and a number of other factors relative to *H. vitripennis* behaviors with the objective of understanding and improving trap efficiency. We have also made some novel discoveries about *H. vitripennis* behavior in response to congeners. We have used the commercially-available Glidden Alkyd Industrial Enamel 4540 Safety Yellow, as the standard color in all tests versus commercial traps, Tangletrap™ (www.Gemplers.com) as the standard sticky substance, and a standard height of 1 m from the ground for trap placement.

We have shown that *H. vitripennis* capture rate may be increased by changing the trap configuration from a flat two-sided trap into a cylinder (tube) shape which apparently samples the entire surrounding 360^0. A comparison of safety yellow mailing tubes 5.1 cm width × 15.2 cm or 30.5 cm length, 7.62 cm width × 15.2 cm or 20.5 cm width, and 10.2 cm width × 15.2 cm length or 20.5 cm length indicated that total GWSS trap capture increased approximately 40-50% in response to each incremental increase in trap size either in width or length. All tube sizes captured significantly more GWSS than the Pherocon AM trap used as the standard in these experiments. We choose a 7.62 cm × 15.2 cm as a standard size of the tube trap because it improves capture rate, is easy to work with and less expensive. In other experiments we tested the tube trap versus the Pherocon AM and the Seabright flat, 2-sided yellow traps as well as versions of the commercial traps configured into a cylinder shape with mixed results. The tube trap always captures numerically greater numbers of GWSS but it was usually only the male leafhoppers that were captured in significantly higher numbers. For example in one test the tube captured 125 (total) leafhoppers (93 males, significantly greater than other treatments, t-test, P<0.001) while 31 (24 males), 25 (17 males) and 37 (23 males) were captured by the Pherocon AM, Seabright and the Seabright cylinder, respectively. In another test, the Pherocon AM captured 10.5 ± 3.5 (mean ± SEM)/trap/sample period, Pherocon AM cylinder captured 12 ± 3.5 and the tube captured 21 ± 3.4. In another test we deployed 10 traps of the tube, the standard Seabright and two Seabright traps together formed into a cylinder with staples to provide the same surface area and relative profile as the tube trap. We placed the traps on 1 m high stakes in a RCB design in a block of large crape myrtle. The Seabright captured a total of 142 GWSS (122 males), the Seabright cylinder 295 (260 males) and the tube captured a total of 389 (337 males) over the duration of the test. All the trap types were significantly different (P<0.0001) using SAS PROC GENMOD and contrast tests to compare means. These results appear to indicate that the cylinder shape provides an advantage but we did not control for the difference in yellow hue inherent in the trap colors. Nevertheless, color does not appear to be the main factor because it is likely that the Seabright cylinder capture rate was in part lower due to our inability to make it completely smooth when we constructed it from two separate traps. However, the Seabright cylinder trap presented a larger target overall because we formed the trap such that the area covered with stickem was equal to that of the tube trap. All things being equal, the tube trap provided a significant increase in capture rate.

Another method to potentially increase trap capture rate and efficiency may be to increase the number of traps used together or to enhance the attraction of the main sticky trap with some additional visual cues. Size matters as explained above. The addition of a host plant clearly increases the number and/or the residence time of responding leafhoppers thereby bringing them in closer contact with the trap. This might function by increasing the active distance of the trap to the leafhoppers, thus in effect sampling more area around the trap. We tested this concept in several ways in several tests with both Seabright and tube traps as follows but found no significant increase in trap capture rate. We placed traps in the field using an randomized complete block (RCB) design with treatments of 1, 2 and 3 traps together 2 m apart. We placed sticky traps ≤ 0.5 m away from another yellow tube without stickem that was 7.6 cm in width but 91 cm in length (bottom 30 cm next to trap and top 60 cm above the sticky trap). We also placed the larger yellow tube without stickem directly above the target sticky trap. These results appear to indicate that GWSS respond directly to traps and do not spend time in any behavior around traps once they respond such as moving down, repeated flying around into the trap, etc. that may be exploited. We also tested a treatment that placed the larger tube without stickem below the target trap (ground -1 m) without significant improvement in trap catch.

GWSS respond strongly to host plant quality and change host plants often. Optimum trap placement relative to host plants was considered as one potential method to improve trap capture rates. Several tests were conducted to investigate GWSS response to traps relative to the presence of a host plant, host plant quality, potential interference of multiple traps upon each other, and the trap distance from a host plant. We placed 30 tube traps on stakes in a 1.22 × 2.4 m grid in a field planting of *Ilex* sp.'Nellie R Stevens' about 1.5-2 m in height on a spacing of 1.22 m between and within 7 rows. Trap height was ca 1.5 m so that traps were even with the tops of the plants. Traps were placed in the planting in every other row such that there were traps in 4 rows so that the outside traps lined up with the inside traps adjacent to them and at a 45^0 angle to the next trap. We compared the trap captures between traps on the out rows vs the inner rows and adjacent traps on the inner rows to determine if there was any difference in trap catch by row placement and the occurrence of nearby traps. This test was completed under unusually high GWSS populations (30-40 GWSS /trap/day) and no significant differences in any factor of trap capture rate were found. This indicates that at least under these "high" populations and trap spacing nearby large plants, traps do not interfere with each other. Figure 1 shows the effect of host plant presence on trap catch using tube traps and a poor host peach vs a good host crape myrtle. Twenty-five container plants each of peach and crape myrtle with adjacent traps and 25 traps without plants were placed in the field in adjacent blocks in a 5 × 5 m grid. GWSS were recorded each day in the morning and afternoon by position either on the plant or trap. The treatments were re-randomized one time per week to remove any positional effects in the field plot. Clearly, the presence and quality of the host plant affected the number of GWSS trapped with the better host crape myrtle attracting more and inducing a higher trap capture rate than peach, the poor host plant, and the traps alone.

In a different study we used tube traps in combination with an array of host plants in containers and compared trap capture between traps with plants within 1 m distance and traps without plants. Host plants used were apple, red oak, 'Tonto' crape myrtle, 'Flordaking', and 'Elberta' peach, redbud, 'Santa Rosa' plum and 'Bradford' pear. We used 7 replicates of each treatment in a RCB design and conducted the test for 38 days. The trap alone captured 135 GWSS and all trap + plant treatments, except for 'Flordaking' peach and 'Santa Rosa' plum which captured less, numerically captured a higher number, on average 47% more, GWSS than the control. Apple, oak, redbud and Bradford pear traps captured statistically significantly higher GWSS than the control ($P<0.05$, LSD). Along with plant quality differences, the distance a trap is placed from a plant may also be a factor potentially affecting GWSS trap capture rates. In another test we placed a 7 × 7 m grid of tube traps 10 m apart centered on a large planting of 'Natchez' crape myrtle of ca. 2 m in height (see other results from this test reported in Northfield et al. 2009). Response of GWSS to traps located at different distances from the crape myrtle plants are shown in Figure 2. A linear relationship inversely related to distance was significant at $P< 0.001$ with an $r^2 = 0.58$. In another test we placed 5 replicates of tube traps and Seabright traps at 1 m and 5 m from large 'Natchez' crape myrtles. In this test GWSS response was: tube 8.7/trap period ± 0.86 (mean ± SEM) at 1 m, and 4.4 ± 0.6 at 5 m, and Seabright 5.2 ± 1.24 at 1 m and 4.6 ± 0.75 at 5 m. The tube at 1 m had significantly higher GWSS ($P < 0.0006$) using SAS Proc GENMOD and contrast statements. Again, the majority of leafhoppers captured were males. Finally, trap efficiency is directly related to how much surface area the trap actually samples termed here as the trap's "active distance". In the case of a cylinder this would be circle of some size around the trap. This parameter is directly related to the size of the trap given that color and height are optimized. We attempted to determine the active distance of the tube trap by placing a set of 5 traps in a configuration where a center trap was surrounded by four other traps in a square around it. We varied the distance of the surrounding square of 4 traps by 6, 8, 10 and 12 m. We also placed a single control trap 35 m away from the treatment trap sets. We used five replicates in a RCB design. Theoretically, the active distance is determined by comparing the treatment capture rates in the center trap to the control trap rates and by comparing the total capture rate between treatments. When the center and control rates are equal then the active distance of the trap is somewhere near this spacing because at lower distances the 4 companion traps interfere with the center trap capture rate which is lower than the control as a result. When the total capture rates per treatment decrease, the trap groupings change at that distance into independent traps rather than acting together (overlapping as in a single visual presentation) as they would at lower distances. Our results were unclear, however, we observed no differences between the center and control traps but total GWSS trap capture rate by treatment did increase linearly from 6, 8 and 10 m and then declined in the 12 m treatment. This suggests that the tube trap may have an active distance of approximately 10 m.

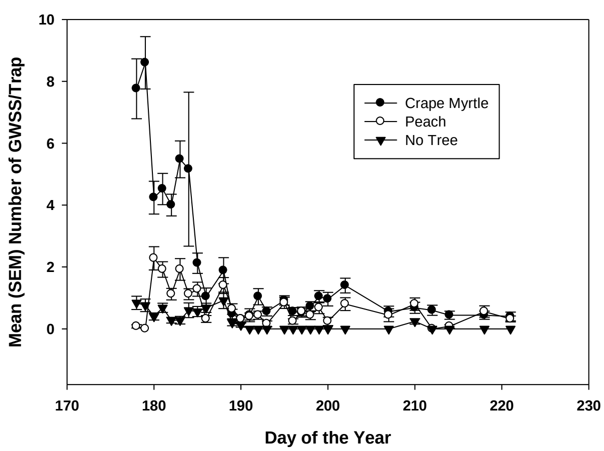

Figure 1: Relationship of GWSS trap capture rate to the presence and absence of host plants: peach and crape myrtle represent poor and good host plants, respectively. Traps used were standard tube traps.

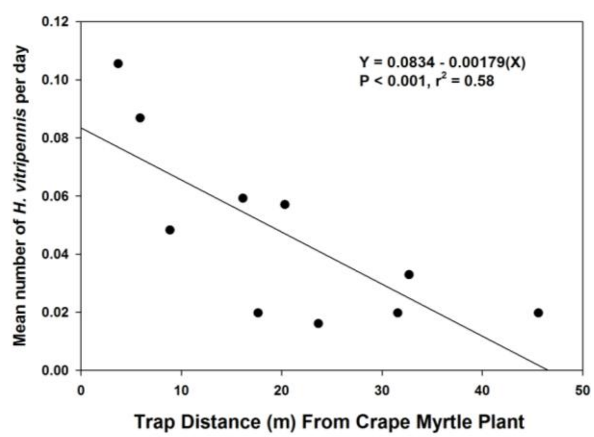

Figure 2: Response of GWSS to traps located at different distances from large 'Natchez' crape myrtle plants. Data points have been normalized by placing them in increments of 10 m centered on the values along the X axis.

The above results, observations of GWSS aggregation behavior in the field on host plants, in response to host plants and on traps led us to examine in depth the response of leafhoppers to traps and to other leafhoppers. The distribution of GWSS landing on traps was investigated with and without other GWSS present. Figure 3 indicates the natural distribution of both sexes of GWSS on the Seabright trap and shows that GWSS tend to aggregate naturally in the lower right hand quadrant. That is in blocks 3-5 (left to right) of the Seabright trap. Figure 4 shows how the GWSS responded to the Seabright trap when there was a dead GWSS cadaver added to the center block on the trap. In this test we allowed only one leafhopper to respond each time to eliminate the confounding effect of previously trapped leafhoppers on the behavior of newly arriving leafhoppers. The presence of a leafhopper carcass shifted the distribution of the arriving leafhoppers to the center area of the trap with males landing at significantly higher numbers in blocks 1 and 2 from the center while females landed in higher numbers in blocks 2 and 4. In another test, GWSS response to an unbaited control Seabright trap was compared to treatments of 1, 2 or 3 GWSS cadavers or 1, 2 or 3 black plastic models similar in size to GWSS added to the center boxes of one side of Seabright traps. Five replicates of each treatment were used in a RCB design. Responding GWSS were recorded by the block number away from the cadaver where they landed as was the number of GWSS that landed on the unbaited side of the trap. Overall the baits increased trap catch by 55% on the baited side versus the unbaited side (data not shown).

Other tests conducted included the addition of a small 15 cm long tree branch with GWSS cadavers on it to tube traps in parallel with the trap orientation and the addition of a similar small branch with cadavers attached to the tube trap in the middle and sticking out at a 45^0 angle away from the trap versus an unbaited control. Neither of these treatments provided significant increase in trap captures. Thus, the response to congeners appears to be a short range landing orientations.

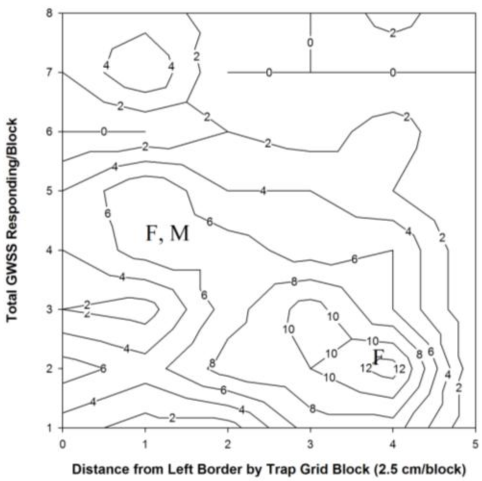

Figure 3: GWSS landing distribution on a Seabright trap without any other GWSS present. M is peak point of males and F indicates female peaks.

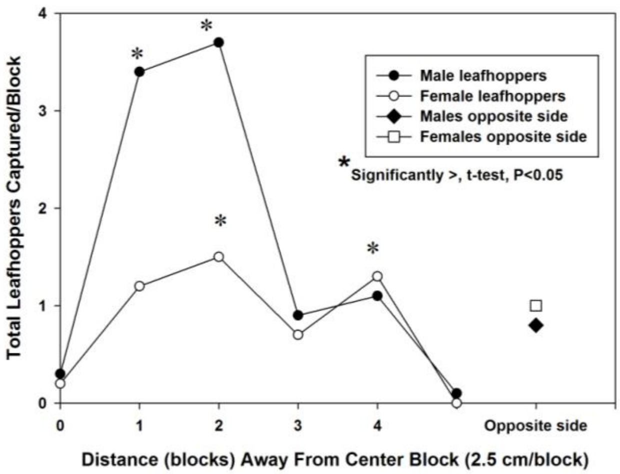

Figure 4: Response of GWSS to Seabright traps baited in the center block with a GWSS cadaver.

We also investigated other visual behaviors of *H. vitripennis* in response to congeners, common heterospecifics and artificial models of these insects. Our objective was to characterize the behaviors and the physical properties of the visual cues involved. *H. vitripennis* adults exhibited highly significant positive responses to congeners, a similar-sized heterospecific species, *Oncometopia nigricans* (Walker), and felt artificial models similar in size to the adult leafhoppers. No significant response was observed to a closely-related leafhopper species, *H. insolita* (Walker) with a smaller body size, or to models of different sizes, shapes and colors other than black and red. Cadavers of leafhoppers placed on a novel and poor host, *L. limii,* attracted and stimulated *H. vitripennis* adults to land preferentially on branches of this poor host plant that contained cadavers. The presence of hidden, live and acoustically-active *H. vitripennis* in cages on a branch with cadavers increased the mean number of responding *H. vitripennis* by 35% on the treated branch vs an untreated control. *H. vitripennis* also landed preferentially on shoots containing conspecific cadavers in the presence of a cadaver of a major predator, *Anolis carolinensis* lizards, or artificial lures resembling spiders. This consistent visual response was independent of both variable bottom-up (host quality) and top-down (predator presence) pressures and suggests for the first time such parsimonious behavior in insects, i.e., sole use of a specific visual cue (conspecifics) as a means to reduce both bottom-up and top-down mortality risks.

CONCLUSIONS:

The experiments conducted in Florida under this grant were all completed in locations with relatively low GWSS populations. These low population conditions typify what would occur under a regulatory/quarantine function when the objective is to detect newly establishing populations when they first likely occur in very low numbers. The collective results of these studies suggest that the detection and monitoring efficiency of trapping of GWSS may be improved in a number of ways. Some are highly practical, whereas others would require a much different approach than the conventional deployment of traps haphazardly in some fashion in the field using a transect or a grid of traps. Use of traps for estimating populations within plants appears to be highly ineffective in most cases with the exception of crops in large acreage blocks where there is little immigration and emigration occurring in the sample location (Castle and Naranjo 2008, Northfield et al. 2009). Changing from the Seabright trap to a cylinder tube trap would improve detection levels but may be impractical relative to costs and logistics required for shipping, moving and storage. Response by GWSS to congeners is novel and may have significant value. We are pursuing this aspect of GWSS behavior. The addition of a printed black silhouette GWSS model to the middle of the Seabright trap may improve its efficiency but this requires more testing under high populations. An increase in trap capture rate may accrue simply from the model's result of shifting the position of GWSS landings on the trap to nearer the center where they are surrounded by larger areas of sticky surface which may decrease their ability to escape the trap. Once leafhoppers arrive on the trap the model effect may lose value. However, from a regulatory perspective this change may be of value, but requires more tests. The attention to trap placement relative to host plant quality, distance from plants and trap size all can be considered for improving trap efficiency and the probability of early detection of low GWSS populations. It does not appear that GWSS exhibit any unusual and exploitable behaviors in either long or short range response to single or multiple trap configurations that may be exploited to improve trap capture rate. The documented use by *H. vitripennis* of congeners and heterospecifics as cues for risk mitigations is extremely novel.

The experimentation for this project has been completed and we are working on the two refereed publications that will result. We have a manuscript in review on the behavioral response to congeners by GWSS and will submit it to a journal in the very near future. The other manuscript is in preparation.

Publications:

Mizell, R., P. C. Andersen, B. V. Brodbeck and W. Hunter. 2010. Behavioral parsimony in the glassy-winged sharpshooter, *Homalodisca vitripennis* (say): visual response to congeners and heterospecifics reduces risks from both bottom-up and top-down forces. In review.

Mizell, R., P. C. Andersen, B. V. Brodbeck and W. Hunter. 2010. Monitoring and detection of glassy-winged sharpshooter, *Homalodisca vitripennis* (say): new trap configurations and associated behaviors. In prep.

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FUNDING AGENCIES

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